REMARKS

Upon entry of this amendment, claims 36-47 and 50 are canceled and claims 1-10, 12-35, 49 and 51-62 are pending. The specification has been amended to correct an obvious typographical error. Claims 1 and 49 have been amended to more clearly recite the invention. Support for the amendment can be found throughout the specification, in particular, at page 3 lines 18-20 and Example 8. Claims 1, 20-22, 28-32 and 49 have been amended to reflect proper antecedent basis. Claims 1 and 21 have been amended to correct typographical errors. Claims 24, 28 and 30 have been amended to correct grammatical errors. Support for new claims 54-62 can be found throughout the specification, for example, at page 44, lines 1-4. Applicants maintain that no new matter has been introduced as a result of the present amendments.

Rejections Under 35 U.S.C. §103

Claims 1-10, 12-35, 49 and 51-53 stand rejected under 35 U.S.C. §103 (a) as being unpatentable over Kim *et al.* (Cancer Treatment Reports, 1987), or Assil *et al.* (Arch. Ophthamol., 1987), or Bonetti *et al.* (Cancer Chemother. Pharmacol., 1994), or Kim *et al.* (5,723,147) or Sankaram *et al.* (5, 766, 627) in view of Lenke *et al.* (5,948,441). The Examiner has stated that all of the primary references teach processes of making multivesicular liposomes, but that they do not teach cross-flow filtration and making a sterile preparation.

The Examiner further has characterized the secondary reference, Lenke *et al.*, as teaching cross-flow filtration used for selection of large quantities of liposomes of a homogeneous, defined size distribution from a heterogeneously-sized population. The Examiner also has stated that Lenke *et al.* teaches various modes of administration and sterilization. The Examiner then reasons that the use of cross-flow filtration would have been an obvious step in addition to the processes of the primary references because Lenke *et al.* teaches its use in the preparation of liposomes and because one of ordinary skill in the art would realize that such preparations should be sterilized. Applicants respectfully disagree for the following reasons.

The present invention relates to a novel process for the production of multivesicular liposomes. Although Lenke *et al.* describes a number of liposome species including unilamellar, multilamellar (MLV), sonicated unilamellar (SUV), plurilamellar (SPLV), frozen and thawed

multilamellar (FATMLV) and reverse-phase evaporation vesicles (REV), Lenke *et al.* does not disclose multivesicular liposomes even though they were known in the art at the time of that invention.

It is well known in the art that multivesicular liposomes are unique particles possessing characteristics that distinguish them from other types of liposomes. These characteristics include relatively massive size, non-concentric vesicle arrangement, and exceptional encapsulation and release properties. These characteristics are the direct result of the distinctive processes used for preparing multivesicular liposomes. By omission of the multivesicular liposome species, Lenke *et al.* fails to disclose the use of cross-flow filtration for the preparation of multivesicular liposomes. Additionally, as discussed more fully below, cross-flow filtration is not useful for size separation of multivesicular liposomes, nor is it employed for that effect in the instant invention. Moreover, contrary to the Examiner's analysis, cross-flow filtration is not necessary as a means for sterilization in the instant invention.

Lenke *et al.* describes the use of cross-flow filtration for use in size separation of pre-formed particles (See '441 at 1:14-16). The liposome preparation methods disclosed by Lenke *et al.* result in the formation of liposomes having an undesirably wide distribution of particle sizes (See '441 at 4:12-13). Lenke *et al.* teaches that "there remains a difficulty in the art of obtaining a homogeneous population of liposomes..." (See '441 at 4:65-67). In stark contrast, no size separation step is required in the claimed processes. The present claims recite processes whereby the size of the multivesicular liposomes is *pre-determined.* The pre-determined liposome sizes are produced according to various process parameters relating to energy input *at the time of liposome formation.* Thus, no post-formation size sorting is required, and the resulting composition can be immediately administered to patients.

The present claims recite sterilization of the liposomes either at the time of formation through use of sterile starting materials, or after cross-flow filtration, prior to filling. Thus, none of the references, either alone or in any combination, teach the ability to control and pre-determine multivesicular liposome size at the liposome formation step of the production process, thereby eliminating the need for post-formation size sorting.

The Examiner has stated that Applicants' [previous] arguments were not persuasive because they were based only upon the Lenke *et al.* reference that teaches cross-flow filtration

and not on the primary references that teach the claimed process except for sterilization. Applicants respectfully maintain that the primary references do not teach how to control the size of the multivesicular liposomes, as is described in the instant invention. Lenke *et al.* does not supply this missing element and in fact teaches away from the control of liposome size at the formation stage, since Lenke *et al.* is directed to methods for post-formation size selection.

The Examiner additionally has stated, "Now it would appear from applicant's arguments that this cross-flow filtration step is not critical at all and that cross-flow filtration is not used as a method of controlling the size distribution of the particles produced according to the inventive method. These arguments appear to be contradictory to applicant's earlier arguments dated 1-16-01." Applicants respectfully disagree with the Examiner's assessment.

In the identified Response, Applicants state, "Cross-flow filtration is used for concentration adjustment of MVL, buffer exchange, and removal of unencapsulated drug. Cross-flow filtration for the preparation of MVL or liposomes gives superior results over the previously described methods of buffer exchange, removal of unencapsulated drug, and concentration adjustment, such as centrifugation and decanting" (See Response paragraph bridging pages 4-5). Thus, it is clear from this statement, as well as from the invention as disclosed throughout the instant specification, that Applicants *never* have asserted that cross-flow filtration is used as a method for controlling particle size distribution in the presently claimed processes.

Applicants' statements quoted above were set forth as merely one way in which to distinguish the invention from the primary references, which do not disclose cross-flow filtration. Applicants in no way expressed that the step of cross-flow filtration was the only way in which the present invention distinguishes over the primary references. In response to Applicants' statements, the Examiner cited Lenke *et al.* as disclosing cross-flow filtration. In Applicants' Response mailed March 28, 2002, Applicants reiterated the statements made in the prior Response, *i.e.*, that cross-flow filtration is used for concentration adjustment of MVL, buffer exchange, and removal of unencapsulated drug. Additionally, Applicants distinguished the present invention from Lenke *et al.* on the basis that the latter teaches cross-flow filtration used for particle size separation. Applicants pointed out that the instant invention relates to a process

for controlling particle size as a function of power input (See Response page 3, paragraph 3) and is not reliant on post-formation size selection.

In order to further illustrate the differences between the Lenke *et al.* use of cross-flow filtration and that of the present invention, Applicant pointed out that even if cross-flow filtration were desirable for size selection according to the present invention (which it is not), 10 μm is the upper limit of the particle sizes for which the Lenke *et al.* cross-flow filtration technique is useful (column 8, lines 53-55). In contrast, multivesicular liposomes are generally larger than 10 μm and therefore cross-flow filtration would not be useful as a means for controlling particle size for these types of very large liposomes. Accordingly, there would have been absolutely no motivation to combine the primary and secondary references, and such combination fails to teach or suggest the present invention.

Thus, Applicants have in no way contradicted their previous statements. Applicants maintain that the primary and secondary references, when taken either alone or in any combination, fail to teach the presently claimed invention which requires control of pre-determined particle size at the time of particle formation. Moreover, Lenke *et al.* fails to disclose the use of cross-flow filtration for use with multivesicular liposomes by not disclosing them along with the other types of liposomes stated in the '441 specification, and because the disclosed size limitations would not be useful for multivesicular liposomes, which are very large. There no motivation to combine the primary and secondary references, and even if combined, the references do not teach or suggest the present invention. Accordingly, Applicants respectfully request reconsideration and removal of this rejection.

Claims 1-10, 12-35, 49 and 51-53 stand rejected under 35 U.S.C. §103 (a) as being unpatentable over Kim *et al.* (Cancer Treatment Reports, 1987), or Assil *et al.* (Arch. Ophthamol., 1987), or Bonetti *et al.* (Cancer Chemother. Pharmacol., 1994), or Kim *et al.* (5,723,147) or Sankaram *et al.* (5, 766, 627) in view of Lenke *et al.* (5,948,441) in further view of Kwasiborski *et al.* (6,033,708), Fenske *et al.* (5,837,282), Mehl, Sr. *et al.* (5,885,260), Castor *et al.* (5,776,486), and Moynihan (5,589,189) by themselves or in combination. The Examiner has stated that each of the tertiary references teach methods of sterilization and that one of ordinary skill in the art would be motivated to prepare the multivesicular liposomes in a sterile

state because the tertiary references each teach methods that involve the production of sterile liposomes. Applicants respectfully disagree for the following reasons.

As previously discussed, there is no motivation to combine the primary and secondary references. Even if such motivation existed, the combination of the primary and secondary references does not teach or suggest the present invention. In particular, none of the references discloses the control of particle size in relation to energy input at the time of liposome formation, with subsequent cross-flow filtration. For reasons already of record, none of the tertiary references teaches or suggests these limitations missing from the primary and secondary references. Moreover, just as the cross-flow filtration step of the instant invention does not relate to particle size selection, nor is it required for sterilization of the resulting composition. Accordingly, Applicants respectfully request reconsideration and removal of this rejection.

Applicants maintain that all pending claims are allowable. Attached is a marked-up version of the changes being made by the current amendment.

As this Response is submitted within two months of the mailing date of the outstanding Office Action, no fees are believed to be due. Please apply any charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

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Version with markings to show changes made

In the specification:

The paragraph beginning at page 17, line 25 has been amended as follows:

-- Sterile filtration of all fluids which enter the MVL production process is essential for an aseptic process, as envisioned in one embodiment of the present invention. Rating of pore sizes of filter membranes is by a nominal rating reflecting the capacity of the membrane to retain [microorganismsof] microorganisms of size represented by specified strains, not by determination of an average pore size and statement of distribution of sizes. Sterilizing filter membranes are those capable of retaining 100% of a culture of 10^7 organisms of a strain of *Pseudomonas diminuta* (ATTC 19146) per square cm of membrane surface under a pressure of not less than 30 psi. Such filter membranes are nominally rated 0.22 μm or 0.2 μm, depending on the manufacturer. Bacterial filter membranes capable of retaining only larger microorganisms (including *Serratia marcescens* (ATTC 14756)) are labeled with a nominal rating of 0.45 μm. Filter membranes used in the present processes are of the 0.2 μm type, and are used in all lines feeding from liquid solution and gas storage tanks to vessels and transfer lines used to manufacture product.--

In the claims:

Claims 36-47 and 50 have been canceled.

Claims 54-62 have been added.

Claims 1, 20-22, 24, 28-32 and 49 have been amended as follows:

--Claim 1. (Twice Amended) A process for preparing a multivesicular [lipid] liposomal particle [formulation] composition, the process comprising:

a) providing a [volume of] first emulsion by mixing [a volume of] a first aqueous phase and [a volume of] a volatile water-immiscible solvent phase, said solvent phase comprising at least one amphipathic lipid and at least one neutral lipid;

b) mixing and emulsifying said first emulsion and [a volume of] a second aqueous phase in a mixer with an energy input to provide [a volume of] a second emulsion, said second emulsion comprising a continuous aqueous phase;

c) removing the volatile water-immiscible solvent from the second emulsion to form [a volume of] a composition of multivesicular liposomal particles of pre-determined size relative to energy input [composition]; and

d) filtering the multivesicular liposomal particle composition by cross-flow filtration,

wherein all steps are carried out under aseptic conditions, and wherein all solutions are sterile filtered prior to use, and wherein the multivesicular liposomal particle composition is immediately suitable for administration into humans.--

--Claim 20. (Amended) The process of claim 16, wherein the back pulse volume is from about 0.01% to about 5% of initial [primary] filtration volume.--

--Claim 21. (Amended) The process of claim 20, wherein the back pulsing volume is from about 0.1% to about 1.0% of initial [primary] filtration volume.--

--Claim 22. (Amended) The process of claim 16, wherein the [primary filtration] filtering is conducted at a retentate back pressure of from about 0 psi to about 10 psi.--

--Claim 24. (Amended) The process of claim 23, wherein the potency adjustment is carried out by secondary filtration.--

--Claim 28. (Amended) The [method] process of claim 27, wherein a first solvent removal step is characterized by an inert gas flow rate [which] that is less than that of a second step.--

--Claim 29. (Amended) The [method] process of claim 28, wherein the gas flow rate of the first solvent removal step is from about 20% to about 50% that of the second step.--

--Claim 30. (Amended) The [method] process of claim 27, wherein a first solvent removal step is characterized by an inert gas flow rate [which] that is greater than that of the second step.--

--Claim 31. (Amended) The [method] process of claim 30, wherein the gas flow rate of the first solvent removal step is from about 120% to about 400% that of the second step.--

--Claim 32. (Amended) The [method] process of claim 28, further comprising a third solvent removal step, wherein the gas flow rate of the third solvent removal step is less than that of the second solvent removal step.--

--Claim 49. (Twice Amended) A process for preparing a multivesicular [lipid] liposomal particle [formulation] composition, the process comprising:

a) providing a [volume of] first emulsion by mixing [a volume of] a first aqueous phase and [a volume of] a volatile water-immiscible solvent phase, said solvent phase comprising at least one amphipathic lipid and at least one neutral lipid;

b) mixing and emulsifying said first emulsion and [a volume of] a second aqueous phase in a mixer with an energy input to provide [a volume of] a second emulsion, said second emulsion comprising a continuous aqueous phase;

c) removing the volatile water-immiscible solvent from the second emulsion to form [a volume of] a composition of multivesicular liposomal particles of pre-determined size relative to energy input [composition]; and

d) filtering the multivesicular liposomal particle composition by cross-flow filtration,

wherein the resulting multivesicular liposomal particle composition is sterilized before filling, and [where] wherein the multivesicular liposomal particle composition is immediately suitable for administration into humans.--